AMENDMENT NO. 1 MARCH 2005
TO
IS 7260 : 1974 SPECIFICATION FOR ETHYL ESTER OF BETA-APO-8′-CAROTENOIC ACID, FOOD GRADE

(*Page* 2, *Table* 1) — Substitute the following for the existing table:

Table 1 Requirements for Ethyl Ester of Beta-Apo-8′-Carotenoic Acid, Food Grade

(*Clause* 2.4)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Appendix	Indian Standard
i)	Purity, as $C_{32}H_{44}O_2$, percent by mass, *Min*	96	A	—
ii)	Sulphated ash, percent by mass, *Max*	0.1	—	Appendix B of IS 6386 : 1971[1)]
iii)	Subsidiary colouring matter, percent by mass, *Max*	3	B	—
iv)	Arsenic (as As), mg/kg, *Max*	3	—	Clause 15 of IS 1699 : 1995[2)]
v)	Lead (as Pb), mg/kg, *Max*	10	—	Clause 15 of IS 1699 : 1995[2)]
vi)	Heavy metals, mg/kg, *Max*	40	—	Clause 16 of IS 1699 : 1995[2)]

[1)] Specification for beta-apo-8′-carotenal, food grade.

[2)] Methods of sampling and test for food colours (*second revision*).

Amend No. 1 to IS 7260 : 1974

(*Page* 4, *Appendix* A) — Insert the following Appendix B after Appendix A:

APPENDIX B

[*Table* 1, *Item* (iii)]

DETERMINATION OF SUBSIDIARY COLOURING MATTERS

B-1 PROCEDURE

B-1.1 Dissolve about 80 mg of sample in 100 ml chloroform. Apply 400 µl of this solution as a streak 2 cm from the bottom of a TLC plate (Silica gel 0.25 mm). Pretreat the thin-layer plate by soaking in a tank with 3 percent KOH in methanol so that it is completely wetted. Then dry the plate for 5 min in the air and activate for 1 h at 110°C in an oven. Let cool over $CaCl_2$ and keep in a desiccator over $CaCl_2$.

B-1.2 Immediately after applying the carotenoid solution to the plate, develop the chromatogram with *n*-hexane/chloroform/ethyl acetate (70 + 20 + 10) in a saturated chamber suitably protected from light, until the solvent front has moved 10 cm above the initial streak. Remove the plate, allow the main part of the solvent to evaporate at room temperature and mark the principal band as well as the bands corresponding to other carotenoids. Remove the silica gel adsorbent that contains the principal band, transfer it to a glass-stoppered 100 ml centrifuge tube and add 40.0 ml chloroform (Solution 1). Separately remove the silica gel of the combined bands corresponding to the other carotenoids, transfer it to a glass stoppered, 50 ml centrifuge tube and add 20.0 ml chloroform (Solution 2). Shake the centrifuge tubes by mechanical means for 10 min and centrifuge for 5 min. Dilute 10.0 ml of Solution 1 to 50.0 ml with chloroform (Solution 3).

B-1.3 Determine, with a suitable spectrophotometer, the absorbances of Solutions 2 and 3 in 1-cm cells at the wavelength maximum in chloroform at about 455 nm, using chloroform as a blank.

B-2 CALCULATION

B-2.1 Carotenoids other than β-apo-8′-carotenoic acid ethyl ester, percent $= \dfrac{A_2 \times 10}{A_3}$

where

A_2 = absorbance of Solution 2, and
A_3 = absorbance of Solution 3.

(FAD 8)

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